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ESTABLISHING LABORATORY STANDARDS FOR BIOLOGICAL FLIGHT EXPERIMENTS

by

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The general objective of this research was to assess the effects of exposure to simulated microgravity on ultrastructural aspects of the contractile system in chicken skeletal muscle cells. This general objective had two specific experimental components: (1) The progression of changes in cell morphology, fusion, and patterns of contractile filament organization in muscle cell cultures grown in hollow fibers in the Clinostat were evaluated, with appropriate controls. (2) To initiate experiments in which muscle cells were grown on the surface of microcarrier beads. The ultimate objective of this second portion of the work is to determine if these beads can be rotated in a bioreactor and thereby obtain a more accurate approximation of the effects of simulated microgravity on differentiated muscle cells.

Thigh muscle from twelve-day broiler chick embryos was removed and disaggregated into individual cells by vortexing the muscle in growth medium on a vortex mixer at maximum speed for 20-30 seconds. The suspension was then filtered to remove connective tissue and bone, and the cells were recovered by centrifugation. Following resuspension in an appropriate volume of growth medium (Eagle's Minimum Essential Medium containing 10% horse serum, 5% chick embryo extract and antibiotics to prevent microbial growth), the cell suspension was injected into a 70 mm long piece of 0.5 mm (inner diameter) XM-80 hollow fiber using a 1cc syringe. Both ends of the fiber were sealed with hot wax, and the fiber was loaded into a glass tube containing growth medium. The fiber was held taut by a spring so that it would always be at the center of rotation of the Clinostat, and the glass tube was then sealed and the entire assembly loaded into the Clinostat.

Alternatively, muscle cells were placed in the presence of 2 x 10^4 microcarrier beads (Pharmacia) per 10 ml of culture medium. These cell suspensions were mixed in 6 cm tissue culture dishes that had not been coated with collagen so that the muscle cells would have a higher affinity for the surface of the beads than for the surface of the polystyrene dishes. The plating density was adjusted such that there were approximately 15 cells per bead after 24 hours. Medium was changed on alternate days and each of the experiments was carried out for up to 14 days. The advantage of growing muscle cells on these beads in comparison to growing cells in the hollow fibers of the Clinostat is that the amount of experimental material is not restricted. Vessels of up to 500 ml could be utilized if necessary, and it would therefore be possible to conduct biochemical measurements of protein metabolism. Such experiments are not currently possible with the Clinostat.

Muscle cell cultures prepared as described above proliferate, fuse and begin to synthesize myofibrillar protein within 2-3 days in culture, and under most experimental conditions they attain a maximum and constant quantity of myofibrillar proteins by approximately 7 days. Because the synthesis rate and the degradation rate must be exactly equal to each other in order to maintain a constant quantity of protein at steady-state, and because perturbations in either synthesis or degradation rates will result in a net change in the

quantity and/or organization pattern of myofibrillar proteins, these cells provide a model for studying the dynamics of muscle protein accumulation and loss.

The Clinostat used for these experiments was designed at MSFC and loaned to us by Dr. Robert Snyder. Briefly, the Clinostat is made up of a culture chamber which rotates about a horizontal axis, and an XM-80 hollow fiber containing cells is mounted in the center of rotation. Operation of the Clinostat in the horizontal position simulates microgravity, and operation in the vertical position serves as a control since the gravity vector is always constant on the cells when the Clinostat is operated in the vertical position. Additional control experiments consisting of cells in hollow fibers lying horizontally in a sealed tube of the same approximate dimensions as the chamber of the Clinostat were also conducted to ensure that a horizontal, non-rotating control was always available. The cells were placed inside the small hollow fiber inside the rotating chamber, the ends were sealed with wax, and the fiber was held taut by the spring-loaded mount. This ensured that the fiber is always held at precisely the center of rotation.

After three days in hollow fibers, muscle cells had initiated the process of fusion and myofibril assembly. Fusion was indicated by the presence of adjacent nuclei in multinucleated myotubes. Additionally, the fact that myofibril assembly had been initiated was suggested by the presence of filamentous material in the general vicinity of the nuclei. In some instances at this stage of differentiation, rudimentary banding patterns were also apparent; however, they lacked the highly organized pattern that is observed later in muscle development or in adult skeletal muscle.

By seven days in hollow fiber cultures, functional and well-aligned myofibrils were present in large quantities.

By fourteen days in culture, several additional changes had occurred in the cells in hollow fibers that were also consistent with those observed in conventional cell cultures. One of the unfortunate consequences of growing skeletal muscle cells in culture in the absence of anchoring connective tissue is that the cells will sometimes contract so vigorously that they detach themselves from the surface and die. Thus, after two weeks in culture many of the remaining cells are those in which no myofibrillar structures are present. Moreover, the percentage of nonmuscle cells will obviously be higher if some of the myotubes have become lost in the medium. Thus, for the purposes of this project, the optimum period of time to examine myofibrillar assembly was approximately one week after establishment of the cultures in the hollow fiber, and most comparisons were made at this time.

In general, cells grew at the same rate, fused at the same time and accumulated myofibrils to the same extent in both horizontally rotated samples and vertically rotated muscle samples. The only potential difference observed was that it seemed that rotation of muscle samples alone has more effect on myofibrillar organization than whether the samples are rotated in the horizontal position (i.e., simulating microgravity) or in the vertical position (i.e., with the gravity vector constant on the cells).

Growth of the muscle cells on the microcarrier beads followed the same general pattern described above, although only light microscopy was employed to observe the pattern of growth. In general, fusion was observed after approximately 3 days in culture, and application of 10^{-7} M fluorodeoxyuridine to inhibit fibroblast growth resulted in a higher percentage of multinucleated muscle cells than was observed in the clinostat cultures. Growth of cells occurred from approximately 15 cells per bead on day 1 to approximately 75 nuclei per bead by 7-8 days. Observation of the myotubes by light microscopy revealed multiple spontaneous contractions after about 5-7 days, and cross-striations were usually observed after approximately 10 days. Although the myotubes stretch across several beads (which tend to form aggregates after several days) and these multiple beads are quite flexible, the contractions are still strong enough to result in detachment of some of the myotubes from the beads. Thus, the number of myotubes decreases in the microcarrier bead cultures as has previously been observed in cultures on the flat surface of polystyrene dishes.

In summary, there seem to be many advantages in using microcarrier beads for eventual experiments on simulating the effects of microgravity on muscle cells. Most significant is the ability to grow large enough numbers of cells to permit the use of rigorous biochemical and molecular biology techniques to examine the possibility that microgravity has a direct, intrinsic effect on atrophy of muscle cells.